PB81-192114

Microbial Bioconcentration of Organic Pollutants from Aquatic Systems A Critical Review

(U.S.) Environmental Research Lab.
Athens, GA

1981

U.S. Department of Commerce
National Technical Information Service
NTIS

F - Doris Paris

TECHNICAL INFORMATION CLEARANCE

Ag 4/13

1. DATE PREPARED	2. LAB/OFFICE DRAFT NO.	3. COPYRIGHT PERMISSION (✓ one)	4. PEER REVIEW CLEARANCE (✓ one)
23 March 1981	ERL-Ath-177	☐ YES ☒ NO ☐ N/A	☒ YES ☐ NO ☐ N/A

5. PRESENT TITLE: Microbial Bioconcentration of Organic Pollutants from Aquatic Systems--A Critical Review

a. TECHNICAL INFORMATION PLAN TITLE AND REFERENCE
not listed Research Committee: F
PE ACULIA

6. AUTHOR, ORGANIZATION, AND ADDRESS
George L. Baughman and Doris F. Paris
Environmental Research Laboratory
USEPA
College Station Road
Athens GA 30613

EPA PROJECT DOCUMENTATION

7. SERIES	8. REPORT DATE	9. CONTRACT/GRANT/IAG NUMBER
EPA-600/J-81-126	Journal Article	

10. TYPE OF MATERIAL (✓ one)
SPECIFY (WHERE NECESSARY)

☐ RESEARCH REPORT
☐ MEETING/PUBLICATION
☐ PROJECT REPORT AND PROJECT SUMMARY
☐ APPLICATIONS GUIDE
☒ JOURNAL PUBLICATION *
☐ SUMMARY/SYNTHESIS
☐ UNPUBLISHED REPORT
☐ RESPONSE REPORT
☐ AUDIO VISUAL

11. PROJECT OFFICER/IN-HOUSE AUTHOR

a. SIGNATURE	b. DATE
George F. Baughman	3/23/81
c. TYPED NAME AND ADDRESS George L. Baughman Athens ERL	d. FTS TELEPHONE NO. 250-3145

12. TECHNICAL INFORMATION (PROGRAM) MANAGER

a. SIGNATURE	b. DATE
Robert C. Ryans	23 March 81
c. TYPED NAME AND ADDRESS Robert C. Ryans Athens ERL	d. FTS TELEPHONE NO. 250-3306

13. COMMENTS

*Published in Critical Reviews in Microbiology. 8(3):250-228, 1981.

PB81 192114

One file

RECEIVED MAR 5 1981

EPA-350(Cin)(8-80)

INSTRUCTIONS

This form must be completed to officially document the clearance of technical material. It certifies that the responsibility of each cognizant official has been met in a manner consistent with ORD Technical Information Policy. Further, it shall be used to provide data essential for subsequent publication processing. It is to be prepared by the Technical Information Manager (TIM) or Technical Information Program Manager (TIPM), where appropriate, and forwarded along with any other necessary papers to the Center for Environmental Research Information (CERI) for action.

INSTRUCTIONS FOR BLOCKS WHICH MAY NOT BE SELF-EXPLANATORY FOLLOW:

3. COPYRIGHT PERMISSION. Originator completes and attaches copyright permission from the holder of the copyright. Copyright permission should indicate that both EPA and NTIS have permission to reproduce.

5a. TECHNICAL INFORMATION PLAN TITLE AND REFERENCE. List title and reference as it appeared in the Technical Information Plan.

7. TECHNICAL SERIES. Specify only one.

10. TYPE OF MATERIAL. Refer to the ORD Technical Information Policy and Guide, March 1980, for descriptions. Where necessary, specify product.

☆ U.S. GOVERNMENT PRINTING OFFICE: 1980 — 657-093/0735

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before comple

1. REPORT NO. EPA-600/J-81-126	2. Journal Article
3 PB81-192114	
4. TITLE AND SUBTITLE Microbial Bioconcentration of Organic Pollutants from Aquatic Systems - A Critical Review	5. REPORT DATE
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) George L. Baughman and Doris F. Paris	8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Environmental Research Laboratory U.S. Environmental Protection Agency College Station Road Athens, GA 30613	10. PROGRAM ELEMENT NO. ACULIA
	11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS U.S. Environmental Protection Agency-Athens GA Environmental Research Laboratory College Station Road Athens, GA 30613	13. TYPE OF REPORT AND PERIOD COVERED Journal Article
	14. SPONSORING AGENCY CODE EPA/600/01

15. SUPPLEMENTARY NOTES
Published in: Critical Reviews in Microbiology, 8(3):205-228, 1981.
Contact: G.L. Baughman, FTS 250-3145

16. ABSTRACT

Bioconcentration of pollutants by aquatic microorganisms has been of interest to environmental scientists for several years because of the position of these organisms in the food chain. Also, similarities between accumulation by microbial species and higher organisms are of interest from a mechanistic viewpoint. Thus, an exhaustive review of the literature has been conducted and the state of knowledge critically evaluated with respect to the kinetics, mechanisms, and magnitude of accumulation. Particular emphasis in the evaluation is directed toward demonstrating that most available data are consistent with a simple mechanism that is "passive" rather than active in the biological sense. Further, it is shown that reliable measurements of the extent of accumulation must take into account both time and biomass concentration. Methods currently in use for experimental measurement and data analysis are discussed to show that many common limitations can be avoided. Some of the more important aspects to be considered involve the use of labeled materials, washed cells, Freundlich isotherm, and molecular structure. In addition, data are presented to show that the octanol-water partition coefficient can be used to predict the potential extent of accumulation.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

PRODUCT OF:
NATIONAL TECHNICAL INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

18. DISTRIBUTION STATEMENT Release to Public	19. SECURITY CLASS *(This Report)* Unclassified	21. NO. OF PAGES 26
	20. SECURITY CLASS *(This page)* Unclassified	22. PRICE

EPA-600/J-81-126
Journal Article

MICROBIAL BIOCONCENTRATION OF ORGANIC POLLUTANTS FROM AQUATIC SYSTEMS — A CRITICAL REVIEW

Authors: **George L. Baughman**
Doris F. Paris
Environmental Research Laboratory
U.S. Environmental Protection Agency
Athens, Georgia

Referee: Robert E. Hodson
Department of Microbiology
University of Georgia
Athens, Georgia

I. INTRODUCTION

Bioaccumulation of aquatic pollutants by higher organisms has long been an area of intensive environmental research. Thus it is surprising to find that so little attention has been given to the segment of biota that forms the base of the food chain and comprises most of the biomass. Possibly this lack of interest stems from the fact that so much attention has been placed on the role of microbial metabolism and transformation. In any event, the presence of persistent pollutants, especially pesticides, in aquatic systems has prompted most bioaccumulation studies and the only previous review, which was by Ware and Roan[1] in 1970.

The review presented here results from an exhaustive search of literature published through early 1978. The important early work on cell permeability by Overton, Collander, and others has been largely excluded, however. Their work was not included because mechanistic considerations are beyond the scope of this review and are only addressed for perspective. Also, these early studies were usually on compounds that are much more soluble (hydrophilic) and readily degradable than the persistent pollutants.

Throughout the review the terms bioaccumulation, accumulation, and bioconcentration are used interchangeably. In every case, these terms are taken to mean the increase in concentration of biomass-associated chemical over the ambient concentration dissolved in water. As will be discussed later, few data are available to indicate whether accumulation occurs internally or on the surface of the cells. The process of accumulation, therefore, is referred to here as sorption or partitioning.

The objective of this review is to offer some reasonable generalizations on the nature of pollutant bioconcentration. In addition, an assessment is made of the data and methodologies through which the generalizations are developed.

II. LOCATION OF SORBATE

Few studies have been carried out for the purpose of determining whether pollutants adsorb on the cell surface or collect internally. From the environmental view, it probably makes little difference because either location results in the reduction of ambient water concentration. Also it seems likely that material in either location is readily available on ingestion by higher organisms. Although most investigators have avoided this question, some have assumed adsorption[2-5] whereas others have referred to absorption

Critical Reviews of
Microbiology, 8(3)
205-228, 1981

or uptake.[6-9] Sugiura et al.[10] have suggested that benzene hexachloride (BHC) accumulation by bacteria occurs via adsorption followed by internal diffusion. Adsorption of colloidal aldrin by flocculant bacteria as a removal mechanism was proposed by Leshinowsky, et al.[11] Sodergren's[12] studies with DDT and *Chlorella* led him to the conclusion that absorption was the dominant process. This conclusion was based on the assumption that adsorbed molecules would be more readily available to the ambient water than the absorbed compound but neither data nor rationale were offered to support this contention.

Several studies are available that indicate the manner in which hydrophobic hydrocarbons may sorb. In one of these, Kennedy et al.[13] showed that an *Acinetobacter* species grown on pentadecane, hexadecane, heptadecane, and hexadec-1-ene could accumulate these compounds against a concentration gradient to form internal "pools" or inclusion bodies. They concluded, therefore, that active transport must be involved. In another study, Kennedy and Finnerty[14] demonstrated that bacterial growth on these hydrocarbons was accompanied by increases in phospholipid and intracytoplasmic membranes.

In the Soviet Union, Poglazova and Meisel[15] studied the polycyclic aromatic hydrocarbon benzo[a]pyrene (BAP) in *Bacillus megatarium* using fluorescent and electron microscopy. They concluded that BAP is localized in lipoprotein membranes such as mesosomes, cytoplasmic membranes, and cell walls. Smith et al.[16] reported a partition coefficient of $(3 - 5) \times 10^5$ for a mixed bacterial population in BAP solution. Current studies, therefore, offer few definitive indications of the site of sorption.

Finally, Ludvic and co-workers have suggested that when the yeast *Candida lipolytica* is grown on hydrocarbons, the hydrocarbons penetrate the cell wall and accumulate at the cytoplasmic membrane.[17] Based on electron microscopy, these authors further suggest pinocytosis as a possible mechanism for transport across the membrane.

In each of the foregoing studies, the organisms were actually grown either on the compounds studied or in their presence under conditions not likely to be found in the environment. Also, the organisms were capable of metabolizing the study compounds, except in the case of BAP for which it was noted that accumulation occurred irrespective of the ability to metabolize. Just how representative these studies are of other organisms and pollutants in the environment is difficult to determine.

III. QUANTITATIVE ASPECTS

The importance of quantitative measurements of bioaccumulation for environmental purposes can hardly be overstated. This stems from the need to predict three factors precisely and reproducibly: (1) extent of bioconcentration of a specified chemical by specified organisms, (2) extent of bioaccumulation of a specified chemical by unstudied organisms, and (3) extent of accumulation of unstudied chemicals by specific organisms. The first of these three factors can be obtained in most cases by direct measurement. The last two, however, represent extrapolations and, therefore, require a theoretical basis for rationale and test. The first factor has received most attention heretofore, but, as we will demonstrate, the latter two, because of their ease of study, hold great promise especially for microorganisms.

The early interest in cell permeability by Overton, Collendar, Danielli, and many others is extremely important to the current view of cell membranes as lipid bilayers. These studies and more recent ones by Diamond and Wright[18,19] and others have presented reasonable attempts to relate cell permeability to molecular structure. Interestingly, Lieb and Stein[20] have shown that permeability of hydrophobic compounds can be treated like diffusion through a soft, non-porous polymer. In such a system, the

permeability is proportional to the membrane-water partition coefficient and inversely related to a fractional power of the molecular weight. For our purposes, we will ignore molecular wegiht effects and note that cell membranes have a bilipid layer structure the permeability of which is proportional to a partition coefficient. Because the pollutants of environmental concern are exceedingly hydrophobic, they can be expected to penetrate the cell membrance rapidly. The reader interested in this subject should consult the articles by Diamond and Wright[18,19] and the excellent books and summaries of this subject.[20-23]

The Gibbs free energy for accumulation in the cell results from an entropy effect that favors movement of the compound from water. This very large escaping tendency (fugacity) of highly hydrophobic organics in the water phase results from a large activity coefficient and accounts for the abnormally low water solubility, high volatility (large Henry's law constant), and perhaps even the membrane structure itself.[24] In this connection, Lakowicz et al.[25] have measured the partition coefficient of the pesticide lindane in phospholipid vessicles and have shown the permeability of the vessicle to be much lower than that of water.

For cells, as well as for liquids, the partition coefficient can be defined as the ratio of equilibrium concentrations in the cell (cell associated) $[P]_{cell}$ and medium (dissolved) $[P]_{H_2O}$.

$$K_B = \frac{[P]_{cell}}{[P]_{H_2O}} \tag{1}$$

It can also be shown from kinetic arguments that the same K_B is equal to the ratio of rate constants that describe the transfer between solution and sorbed phase if $[P]_{cell}$ is directly in equilibrium with pollutant in solution (Equation 2).

$$[P]_{H_2O} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} [P]_{cell} \tag{2}$$

$$K_B = \frac{k_1}{k_{-1}} \tag{3}$$

Thus Equations 1 and 3 provide a mathematical statement of the definition of bioaccumulation given earlier if metabolism of the solute is negligible.

In addition to Equation 1, Equation 4 has also often been used. In 1909 Freundlich proposed this equation

$$[P]_{cell} = K_f [P]_{H_2O}^{1/n} \tag{4}$$

to describe adsorption phenomena. Its use in microbial accumulation studies probably stems from the previously noted lack of distinction between adsorption and absorption.

Two features of the Freundlich equation are worth noting. First, the value of the constant, n, 1/n approaches unity for many systems at low concentrations. Second, when 1/n is unity, the Freundlich constant, K_f, is equivalent to K_B and one obtains the same result as with Equation 1.

IV. DISCUSSION OF LITERATURE

Table 1 summarizes the data resulting from the literature search. The table indicates

whether K_a or K_f was measured and whether hydrogen ion activity (pH), viability, or time were factors considered in the studies. These factors and others are considered in more detail below.

A. Effect of pH

Only a few workers[28,37,50] have examined the effect of pH on microbial partitioning. Chacko and Lockwood[28] looked at mycelial accumulation of DDT and dieldrin by *Streptomyces venezuela* and *Trichoderma viride* over the pH range of 3.0 to 11.0. They found that *S. venezuela* took up 90 to 100% of both pesticides at pH 5.5, 8.0, and 11.0. At pH 3.0, the accumulation dropped to 75% and 40% for dieldrin and DDT, respectively. On the other hand, *T. viride* accumulated 90 to 100% of both compounds at pH 3.0, 5.5, and 8.0. At pH 11.0, accumulations dropped to 80% for dieldrin and 52% for DDT. All of these changes are probably within experimental error.

Grimes and Morrison[37] looked at sorption of α and β chlordane and lindane by three bacterial species over the pH range of 6 to 8. They report pH 7 to be optimum but the change in concentration that they observed did not exceed about a factor of two.

Valentine and Bingham[50] in examining the influence of pH on algal accumulation of atrazine found little effect over the range of pH 6.1 to 8.3.

The above results indicative of small pH effects are entirely consistent with expectation if hydrophobicity of the compounds provides the driving force. If protonation or other reactions of the molecule can occur in the pH range of interest, however, then large effects should be anticipated.

B. Effect of Equilibration Time

Because accumulation, or K_a, is an equilibrium parameter, it is necessary to establish that equilibrium has been attained before measurements are made. No detailed kinetic studies are available in the literature but many workers have made measurements over time to establish the lack of temporal variation in their data.[11,12,26-28,32,39,41,44] In general it appears that equilibrium is reached in a matter of minutes to a few hours. A similar result has been found with dead yeast cells and anthracene.[2] The only reported exception to this is given by Reinert[43] in his study of the alga *Scenedesmus obliquus* and dieldrin in which he reports equilibrium times of 24 to 36 hr.

Using methoxychlor and toxaphene, Paris et al.[40-42] found that the time required for equilibrium was greater for a fungus than for an alga and that bacteria equilibrated much faster than either. This finding is consistent with expectation based simply on size of the cells and in keeping with the overall impression one has on reading the papers cited in Table 1. Unfortunately, reliable comparisons between the various studies shown in Table 1 are not possible because kinetic data are absent.

Other, still tenuous data, indicate the manner in which compound properties influence equilibration time. Paris et al.[41] found that equilibrium was attained faster between microorganisms (bacteria, *Chlorella* and *Apergillus*) and toxaphene than with methoxychlor, which is much less soluble. A similar order between solubility and time exists in the data of Neudorf and Khan[38] for a freshwater alga exposed to DDT, dieldrin, and photodieldrin. Chacko and Lockwood[28] report that fungal mycelia equilibrate faster with dieldrin than with the less soluble DDT. Rice and Sikka[44,45] found the same order with marine algae.

An inverse relationship between equilibration time and water solubility is inconsistent with the early work on cell permeability. It will be recalled that cell permeability was found to be proportional to solvent-water partition coefficients, which are known to be inversely related to solubility. There are two ready explanations for this apparent discrepancy. First, equilibration time is a system specific property and valid compari-

Table 1
K_a AND K_r FOR VARIOUS MICROORGANISMS AND VARIOUS POLLUTANTS[a]

Compound	Organisms	K_a[b]	K_r[b]	Viable	Dead	Time[c]	Ref.
αHCH	*Chlorella pyrenoidosa*	(153—267)		X	X	15 min	26
αHCH	*Chlamydomonas* sp.	2,700(310)	(330)	X	X	30 min	27
	Dunaliella sp.	1,500		X			
DDT	*Mucor ramannianus*			X			28
	Giomorella cingulata			X			
	Trichoderma viride			X		4 hr	
	Streptomyces lavendulae			X			
	Streptomyces griseus			X			
	Streptomyces Venezuelae			X		4 hr	
	Bacillus subtilis			X			
	Serratia marcescens			X			
	Agrobacterium tumefaciens						
Dieldrin	*Mucor ramannianus*			X			
	Giomorella comgilata			X			
	Trichoderma viride			X		15 min	
Dieldrin	*Streptomyces lavendulae*			X			28
	Streptomyces griseus			X			
	Streptomyces venezuelae			X	X	15 min	
	Bacillus subtilis			X			
	Serratia marcescens			X			
	Agrobacterium tumegaciens		X				
PCB	*Colpidium campylum*	2,500—3,500[d]		X		43 hr	29
DDE	Zooplankton	$(2.9 - 6.4) \times 10^{4}$[e]		X			30
Lindane	Zooplankton	170—448[e]		X			
Anthracene	Yeast		2.48×10^{4}		X	1 min	2
DDT	Algae	1.3×10^{4}[f]					3
Mirex	*Chlorococcum* sp.	7,300[g]					31
	Chlamydomonas sp.	3,200[g]					
Mirex	*Dunaliella tertiolecta*	4,100[g]					31
	Thallasidsira pseudomana	5,000[g]					
Methoxychlor	*Aerobacter aerogenes*			X	X	30 min	32
	Bacillus subtilis			X	X	30 min	

Table 1 (continued)
K_a AND K_r FOR VARIOUS MIIROORGANISMS AND VARIOUS POLLUTANTS[a]

Compound	Organisms	K_a[b]	K_r[b]	Viable	Dead	Time[c]	Ref.
DDT	*Aerobacter aerogenes*			X	X	30 min	
	Bacillus subtilis			X	X	30 min	
DDT	*Cylindrotheca closterium*	265		X		3 weeks	33
Pentadecane	*Acinetobacter* sp.			X			14
Hexadecane	*Acinetobacter* sp.			X			13
Heptadecane	*Acinetobacter* sp.			X			
Hexadec-1-ene	*Acinetobacter* sp.			X			
Lindane	*Euglena* sp.		X	X			34
	Chlorella sp.		X	X			
Parathion	*Euglena* sp.		X	X			34
Dieldrin, PCNB and DDT	*Aphanomyces euteiches*			X	?		35
	Fusarium solani			X	?		
	Pythium ultinum			X			
	Rhizoctonia solani			X	X		
Dieldrin	*Streptomyces albus*			X	?		
	Streptomyces aureofaciens			X	?		
	Streptomyces griseus			X	?		
	Streptomyces viridochromogenes			X	?		
Aldrin	Lake bacteria	625					11
2,5,2′-Trichlorobiphenyl	*Oedogoium cardiacum*	7,315					36
2,5,2′,5′-Tetrachlorobiphenyl	*Oedogonium cardiacum*	17,997					36
2,5,2′,4′,5′-Pentachlorobiphenyl	*Oedogonium cardiacum*	5,464					
DDE	*Oedogonium cardiacum*	11,251					
α-Chlordane	*Achromobacter delicatulus*	X		X			37
β-Chlordane	*Bacillus megatarium*	X		X			
Dieldrin	*Baccilus subtilis*	X		X			
Heptachlor epoxide	*Caulobacter vibrioides*	X		X			
Lindane	*Chromobacterium violaceum*	X		X			

	Enterobacter aerogener	X		X		15 min	
	Escherichia coli	X		X			
	Zoogloea ramigera	X		X		15 min	
	Micrococcus roseus	X		X			
	Pseudomonas aeruginosa	X		X			
	Pseudomonas fluorescens	X		X			
	Pseudomonas putida	X		X			
	Sphaerotilus natans	X		X			
DDT	*Ankistrodesmus amalloides*	X		X		120 min	38
Dieldrin	*Ankistrodusmus amalloides*	X		X		30—60 min	
Photodieldrin		X		X		30 min	
Methoxychlor	*Chlorella* sp.						39
2,4-DBE	*Nitzschia* sp.						
Carbaryl	*Monoraphidium* sp.						
	Anacystis nidulans						
Toxaphene	*Bacillus subtilis*		3.4×10^3	X	X	30 min	40
	Flavobacterium harrisonii		5.2×10^3	X	X	30 min	
	Aspergillus sp.		1.7×10^4	X	X	10 min	
	Chlorella pyrenoidosa		1.1×10^4	X	X	2 hr	
Toxaphene	*Bacillus subtilis*		3.4×10^3	X	X	30 min	41
	Flavobacterium harrisonii		5.2×10^3	X	X	30 min	
	Aspergillus sp.		1.7×10^4	X	X	10 min	
	Chlorella pyrenoidosa		1.1×10^4	X	X	2 hr	
Methoxychlor	*Bacillus subtilis*		4.8×10^4	X	X	30 min	41
	Flavobacterium harrisonii		1.2×10^3	X	X	30 min	
	Aspergillus sp.		5.2×10^3	X	X	16 hr	
	Chlorella pyrenoidosa		8.4×10^3	X	X	30 min	
Methoxychlor	*Bacillus subtilis*		4.8×10^4	X	X	30 min	42
	Flavobacterium harrisonii		1.2×10^3	X	X	30 min	
	Aspergillus sp.		5.2×10^3	X	X	16 hr	
	Chlorella pyrenoidosa		8.4×10^3	X	X	30 min	
Dieldrin	*Scenedesmus obliquus*		1.3×10^3	X		16 hr	43
DDT	*Skeletonema costatum*			X	X		44
	Cyclotella nana			X	X		
	Isochrysis galbana			X	X		
	Olisthodiscus luteus			X	X		

Table 1 (continued)
K_s AND K_r FOR VARIOUS MICROORGANISMS AND VARIOUS POLLUTANTS[a]

Compound	Organisms	K_s[b]	K_r[b]	Viable	Dead	Time[c]	Ref.
	Amphidinium carteri			X	X		
	Tetraselmis chuii			X	X	2 hr	
Dieldrin	*Skeletonema costatum*			X		1 hr	45
	Cyclotella nana			X		1 hr	
	Isochrysis galbana			X		1 hr	
	Olisthodiscus luteus			X		1 hr	
	Amphidinium carteri			X		1 hr	
	Tetraselmis chuii			X		1 hr	
Dieldrin	Benthic algae	$10^3 - 3 \times 10^5$		X	X		46
C^{14}-Chlordane	*Oedogonium cardiacum*	9.8×10^{4h}		X			47
Chlordane	*Oedogonium cardiacum*	4.9×10^{4h}		X			
C^{14}-Toxaphene	*Oedogonium cardiacum*	6.9×10^{3h}		X			
Toxaphene	*Oedogonium cardiacum*	1.4×10^{4h}	X				
Aldrin	*Anabaena cylindrica*	$(1.3 \pm 0.3) \times 10^3$		X			48
	Acacystis nidulans	$(9.9 \pm 1.8) \times 10^2$		X			
	Nostoc muscorum	Converted to dieldrin		X			
Dieldrin	*Anabaena cylindrica*	$(2.0 \pm 0.4) \times 10^2$		X			48
	Anacystis nidulans	$(5.0 \pm 1.3) \times 10^2$		X			
	Nostoc muscorum	$(1.8 \pm 0.3) \times 10^3$		X			
DDT	*Rhodotorulus solani*	900		X			
	Rhodotorulus solani	1,070			X		
	Alfalfa tissue	1,050		X			
	Alfalfa tissue	1,360			X		
Benz[*a*]anthracene	Bacteria	$(3.6 \pm 0.3) \times 10^4$		X			16
	Bacteria	$(9.9 \pm 0.7) \times 10^4$			X		
Benzo[*a*]pyrene	Bacteria	$(3 - 5) \times 10^{5r}$			X		
Benzo[*f*]quinoline	Bacteria	149		X			
	Bacteria (heat killed)	540			X		
7H-Dibenzo[*c,g*]-Carbazole	Bacteria	$(8.4 \pm 1.6) \times 10^4$		X			
Benzo[*b*]thiophene	Bacteria	126		X			

	Bacteria (heat killed)	280			X		
Dibenzothiophene	Bacteria	1.52×10^3		X			16
	Bacteria (heat killed)	2.90×10^3			X		
Methyl parathion	Bacteria (25°C)	485		X			
	Bacteria (2°C)	221		X	X		
	Bacteria (heat killed 25°C)				X		
Mirex	Bacteria	4.0×10^5		X			
C^{14}-DDT	*Chlorella* sp.			X	X	15 sec	12
BHC residue	Bacteria		4.2×10^3	X	X		10
	Bacteria		3.7×10^2	X	X		
	Bacteria		2.6×10^3	X	X		
BHC	*Fusarium roseum*			X	X	1 hr	6
Tetrachlorobiphenyl	*Chlorella pyrenoidosa*	4.8×10^3		X			49
	Chlorella pyrenoidosa	1.1×10^4			X		
Hexachlorobiphenyl	*Chlorella pyrenoidosa*	1×10^4		X			49
	Chlorella pyrenoidosa	2.4×10^4			X		
Octachlorobiphenyl	*Chlorella pyrenoidosa*	1.1×10^4					
Decachlorobiphenyl	*Chlorella pyrenoidosa*	8.7×10^3					
Atrazine	*Scenedesmus quadricauda*	200[f]		X		6 hr	50
	Chlorella pyrenoidosa	150[f]		X			
	Euglena gracilis	40[f]		X		6 hr	
	Chlamydomonas reinhardtii	100[f]		X		6 hr	
Dieldrin	*Microcystis aeruginosa*			X		30 min	51
p,p′-DDT	*Anabaena cylindrica*			X		30 min	
Aldrin	*Scenedesmus quadricauda*			X		30 min	
Endrin	*Oedogonium* sp.			X		30 min	
Lindane	*Saccharomyces cerevisiae*		$(1.4 \pm 0.1) \times 10^2$	X		30 min	5
	Saccharomyces cerevisiae		$(2.2 \pm 0.4) \times 10^2$		X		
Dieldrin	*Saccharomyces cerevisiae*		$(1.1 \pm 0.3) \times 10^3$	X		30 min	
	Saccharomyces cerevisiae		$(4.6 \pm 0.9) \times 10^3$		X		
Chlorinated naphthalenes	*Chlorococcum* sp.			X			7
Dieldrin	*Chlorella pyrenoidosa*			X			8
Hydrocarbons (hexadecane)	*Candida lipolytica*			X			17

Table 1 (continued)
K_a AND K_r FOR VARIOUS MICROORGANISMS AND VARIOUS POLLUTANTS[a]

Compound	Organisms	K_a[b]	K_r[b]	Viable	Dead	Time[c]	Ref.
^{14}C-DDT	*Syracosphaera carterae*	2.5×10^4		X			9
	Amphidinium carteri	8.0×10^4		X			
	Thalassiosira fluviatilus	2.5×10^4		X			
Benzo[a]pyrene	*Pseudomonas aeruginosa*			X			52
	Escherichia coli			X			
Aldrin dieldrin	*Oedogonium* sp.	4×10^4					53
	Oedogonium sp.	3.6×10^3					
Dieldrin	*Oedogonium* sp.	4.6×10^2					
Endrin	*Oedogonium* sp.	4.6×10^3					
Mirex	*Oedogonium* sp.	6.0×10^2					
Hexahclorobenzene	*Oedogonium* sp.	5.2×10^2					
Benzo[a]pyrene	*Escherichia coli*			X	X	4 hr	54
	Saccharomyces cerevisiae			X			
2,4,5,2′,5′-pentachlorobiphenyl	*Guinardia flaccida*	$(4.6 \pm 0.9) \times 10^5$		X			55
	Lithodesmium undulatum	$(3.6 \pm 1.0) \times 10^5$		X			
	Ditylum brightwelli	$(8.7 \pm 1.0) \times 10^5$		X			
	Luderia borealis	$(1.4 \pm 0.4) \times 10^6$		X			

[a] X indicates that authors made observations on effect of viability or death.
[b] K_a and K_r are given if available on weight-weight.
[c] Time given is that stated by authors as required for equilibrium.
[d] Volume basis.
[e] After first day.
[f] Estimated from authors' data.
[g] Washed cells.
[h] Mixture of organisms.

sons can only be made if all system conditions are identical, which was not the case here, e.g., compounds were present at different molar concentration. Second, it is possible that a different mechanism is operative here. This possibility is not unreasonable because the early permeability studies used compounds that have partition coefficients orders of magnitude smaller than the ones cited.

It is interesting to note that, in the analogous case of accumulation by sediments, an inverse relationship exists between equilibration time and partition coefficient.[56] Such a relationship is expected for diffusion limited transport when significant partitioning occurs between water and the medium in which diffusion takes place. This has been suggested by Sugiura et al.[10]

C. Effect of Cell Viability

In this review nearly every paper included has at least some data resulting from observations of sorption by dead organisms. In most cases, the data are simply comparisons rather than measured K_ps. In almost all studies, the conclusion has been that sorption occurs at least to the same extent or usually slightly greater in dead cells than in live cells.[16,26-28,32,41,44,57]

The most detailed and careful quantitative studies of partitioning by microbial cells have been made with yeast by Voerman and Tammes[5] and by Herbes.[2] In both of these studies, the dead cells were heat killed and Voerman and Tammes[5] showed the K_f to increase by a factor of 4 to 6 for the dead cells. Unfortunately, Herbes[2] made no measurements on viable cells. Rice and Sikka[44] found that several species of heat or mercuric chloride ($HgCl_2$) killed algae gave similar results to viable cells except in the case of *Amphidinium carteri* for which sorption increased 1.9 to 2.5 times. Sodergren[12] reported sorption to be similar between live and dead algae (killed by $HgCl_2$) both with respect to rapid uptake and extent of accumulation. In a study by Ueyama et al.[6] cells of *Fusarium roseum* were killed by 70% ethyl alcohol, boiling, $HgCl_2$, and formaldehyde. In each case results were similar to those with viable cells. This finding has led most investigators to conclude that partitioning[37,49,58] or nonmetabolic processes[28,41] are involved. Ko and Lockwood,[57] however, assumed that their chlorinated compounds were being accumulated against a concentration gradient in soil systems and therefore suggested an active transport mechanism.

It is interesting to note that partitioning by biological material both live and dead is not peculiar to microorganisms. Cox[59] reported similar results for initial uptake of DDT by a euphausiid shrimp. Shin et al.[4] found this behavior with alfalfa tissue and with the fungus *Rhodotorulus solani*. Finally, Rose and McIntire[46] report a qualitatively similar result with living and dried *Spirogyra* suspended in laboratory streams containing DDT. Partitioning by higher organisms such as fish is, of course, well known.

D. Choice of Phenomenonological Equation

As mentioned earlier, either the partition equation (Equation 1) or the Freundlich equation (Equation 4) can be used to describe accumulation data. Both equations have often been used (Table 1), but only one paper[27] has presented the results from both equations. In no case have authors who used the Freundlich equation shown it to be superior on statistical grounds. In fact, in those cases where the most data exist,[2,5,10,27,41,60,61] the authors have found the Freundlich 1/n to be approximately 1.[2,5,41] In the other cases, inadequate data are available in the paper to determine whether the Freundlich equation is more suitable than a simple partition coefficient. Certainly, any three-parameter equation will fit data better than a similiar two-parameter equation. Consequently, the principle of OCCAM's razor indicates that unless we

can justify the more complex Freundlich equation, the simpler partitioning equation should be used.

One might question whether these equations are expected to hold under conditions where more than one solute is present. This was addressed in the study by Voerman and Tammes[5] using yeast, lindane, and dieldrin. As would be expected for a purely physical process, each compound was found to sorb independently.

E. Relationship between Compound Properties and K_p

It is well known[36,62] that an inverse relationship appears to exist between water solubility and extent of bioaccumulation in higher organisms. Similar behavior is observed with halogenated pesticides and algae[3,40,45] and with bacteria.[10,37,40] None of these studies include more than three compounds for which the solubilities are known, however.

The octanol-water partition coefficient (K_{ow}) has been found to be a useful parameter in predicting bioaccumulation in higher organisms. This association has apparently not been attempted for microorganisms but the relationship between K_p and K_{ow} should be linear. The linearity of these data from several authors (Table 2 and Figure 1) is remarkable considering that the Ks span several orders of magnitude and that many of the points represent average values for mixed populations or several species.

V. IMPLICATIONS FOR METHODOLOGY

The foregoing leads to several conclusions concerning the measurement of biological accumulation by microorganisms. We will assume that it is desirable to precisely determine the accumulation factor (K_p) as a fundamental parameter having significance in most biological systems.

Either kinetic or equilibrium methods can be used for measurement as indicated by Equations 1 and 3. The rapid rate of sorption by microorganisms, however, makes kinetic measurements difficult, which undoubtedly accounts for their absence from literature. Spectroscopic methods such as those described by Lakowicz et al.[25] hold considerable promise particularly if coupled with conventional equilibrium measurements.

Quantitative measurement requires at the very minimum statistical treatment of the data. It is a serious defect in the studies reviewed that no more than four or five authors have provided any statistical treatment whatsoever. Nor are sufficient raw data given to permit a reader to make statistical analyses.

Another factor to be considered is the units in which K_p is to be reported. When concentration in each phase is given in units of mass of solute per mass of phase, the K_p has units of mass of water per mass of biota. Reproducibility demands a description of the standard condition of the biological phase for which the mass is determined. Dry weight is probably the simplest and most straightforward, provided drying conditions are given. Often, wet weight or cell numbers are given but use of such units does not lend itself as well to extrapolation or comparision.

Because bioaccumulation of highly hydrophobic compounds is believed to be related to lipid content, variation caused by different species might be reduced by relating K_p to lipid content. Canton et al.[27] have studied sorption of α-hexachlorocyclohexane by the algae *Chlamydomonas* and *Dunaliella*. They found that *Chlamydomonas* and *Dunaliella* had K_ps of 2700 and 1500, respectively, on a dry weight basis. When the K_ps were normalized to lipid content, the values were 12,000 and 13,000. Other data relevant to lipid content are in papers by Johnson and Kennedy[32] and Paris et al.[42] In neither study did the measured K_ps follow the expected lipid contents of Gram negative and Gram positive organisms.

A. Mass Balance of Sorbate

It is possible to rigorously defend the accuracy of bioaccumulation measurments only when the concentration of sorbate is determined in both water and biomass. The method most often used is to add a known quantity of solute, measure the amount in one phase, and determine the amount in the other phase by difference. When this "difference" method is used, appreciable errors can be caused by sorption on filters or glassware, adherence of organism to glassware, or rapid volatilization. The problem of metabolism is even more important. This might be anticipated when working with bacteria or fungi, but it is often not expected with algae.[48] Two algal studies[8,39] have reported, however that sorbed compounds become more difficult to recover with time. Also, metabolism of compounds like DDT,[39,44] amitrole,[50] and aldrin[48] has been observed and points up the potential hazard of ignoring mass balance. This is particularly important when the transformation is extensive or rapid.[39,48]

B. Phase Separation

The biological phase is typically separated from the water by either filtration or centrifugation; both methods have been used by many workers. If properly performed, either method can probably give good results.

The major difficulty with filtration is that most membrane filters are highly hydrophobic and often have a large mass compared to the biomass. Consequently, they can sorb large quantities of solute very efficiently. This results in a greatly reduced filtrate concentration ($[P]_{H_2O}$) and a serious overestimate in the magnitude of K_B if "difference" methods are used. The efficiency of filtration as a removal mechanism is sufficient to permit its suggestion as a recovery technique for compounds like PCBs and DDT[35] in analysis of water. This does not mean that filtration cannot sometimes be used effectively. It should, however, place a heavy burden of proof on the researcher.

Centrifugation is more straightforward if adequatate sample is available. Questions arising in this case are

1. What is the error associated with suspended biomass?
2. How can the cells be washed (rinsed)?
3. Is volatilization important?

Some degree of error is always associated with the finite amount of biomass that is not pelleted or that becomes resuspended on decanting the supernatant solution. This results from the fact that most analytical techniques will measure everything in the aqueous phase as if it were dissolved.

The contribution from suspended material to the true value is readily calculated by modifying Equation 1.

$$\text{Aqueous fraction dissolved} = \frac{V}{V + (m)\, fK_B} = \frac{1}{1 + \left(\frac{m}{V}\right) fK_B} \quad (5)$$

where V is the mass of water, f is the fraction of total biomass suspended and (m) is the total biomass in the system. A quick calculation shows that if the fraction of biomass suspended does not exceed 0.1, the fraction of solute dissolved in the aqueous phase will always exceed 0.90 unless the K_B is greater than the reciprocal of the total biomass concentration. Because it is unlikely that 10% of the biomass will remain suspended, proper arrangement of biomass/water ratios will usually make it unnecessary to wash the cells. It is significant that nearly all of the work reviewed here involved washing cells, a potential problem that deserves further examination for other reasons outlined below.

Table 2
BACTERIAL SORPTION (K_B) AND OCTANOL-WATER (K_{ow}) PARTITION COEFFICIENTS OF SEVERAL ORGANIC COMPOUNDS

Compound	K_B	Ref.	K_{ow}	Ref.
Benzo (*a*) anthracene	3.6×10^4	16	4.1×10^5	63*
Benzo (*a*) pyrene	3.4×10^5	16	2.2×10^6	63*
Benzo (*f*) quinoline	1.5×10^2	16	1.6×10^3	63*
Benzo (*b*) thiophene	1.3×10^2	16	1.3×10^{3}*	63
Dibenzocarbazole	8.7×10^4	16	5.6×10^5	63*
Dibenzothiophene	1.5×10^3	16	1.3×10^4	63*
Methylparathion	4.9×10^2	16	6.5×10^3 (ethyl)	62
Methoxychlor	2.5×10^4	42	1.2×10^5	64
Mirex	4.0×10^5	16	7.8×10^6	69
PCBs				
Aroclor 1016	6.1×10^3	60	2.4×10^4	51
Aroclor 1242	5.2×10^3	60	1.3×10^4	60
Phenanthrene	6.3×10^3	68	3.7×10^4	64
Pyrene	2.7×10^4	68	1.5×10^5	64
Toxaphene	4.3×10^3	40	3.3×10^3	40

* Calculated from Reference 63.

When cells in equilibrium with a medium having solute concentration, $[P]_e$, are exposed to a medium of different concentration, the equilibrium should shift according to Equation 1. The very existence of partitioning implies such behavior, but several workers have also measured the desorption and obtained results similar to the sorption case.[10,16,40,42] Smith et al.[16] actually measured the K_B for both sorption and desorption with the result shown in Table 3.

Grimes and Morrison[37] and Voerman and Tammes[5] have examined the desorption of chlorinated pesticides by centrifuging equilibrated cells, discarding the supernatants and resuspending in an equal volume of pesticide-free medium.

This general procedure or some variant has often been used[32] as a basis for conclusions about the strength or reversibility of sorption. But even if there are no kinetic limitations, detectable desorption cannot occur unless $(m/V)K_B$ is small enough to permit a concentration change greater than experimental error. This stems from the fact that the fraction of a solute in each phase is a function only of the ratio of the masses of the two phases (Equation 6).

$$\text{Fraction desorbed} = \frac{V}{mK_B + V} = \frac{1}{\left(\frac{m}{V}\right) K_B + 1} \qquad (6)$$

Such conditions were used by Grimes and Morrison[37] but probably would not allow detectable desorption to occur because their sorption results can be used to show that the fraction of α-chlordane unsorbed ranged from less than 1% to only 72% for the three organisms studied. Their conclusion about the difficulty of desorption is thus based on an observation that is an artifact of the experimental conditions.

The study by Grimes and Morrison[37] did show that the chlordane equilibrium was not significantly changed by deionized water, 0.5% acetone, or phosphate buffer (Figure 2). This observation is consistent with a partitioning mechanism because the solubility, partition coefficient, or other properties related to the activity of highly hydro-

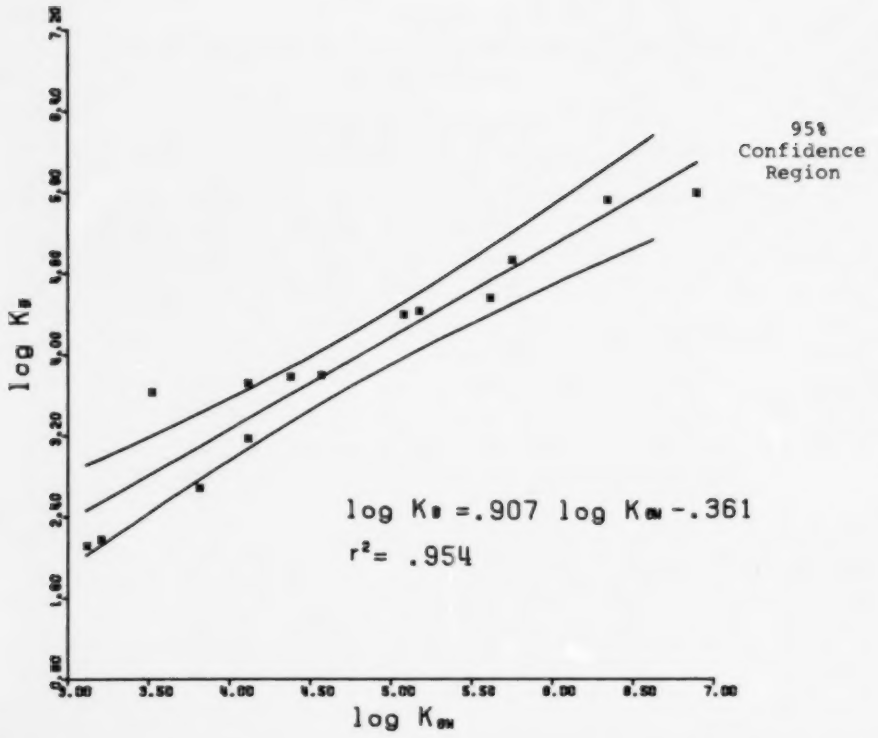

FIGURE 1. Microbial sorption (log K_s) vs. octanol-water partition coefficients (log K_{ow}) for several organic compounds.

phobic compounds should be only slightly influenced by the presence of dilute organic or inorganic solutes.

The desorption study by Voerman and Tammes[5] is interesting because it represents sucessive extraction of the organisms by fresh solution. This situation has been dealt with many times for liquid-liquid extractions and can be described by the general equation

$$X_n = X_0\left(\frac{m}{\frac{V}{K_B}+m}\right)^n = X_0\left(\frac{1}{\frac{V}{mK_B}+1}\right)^n \qquad (7)$$

where X_n is the amount of solute remaining in the biomass after n extractions, X_0 is the initial amount present in the biomass, and m and V are as previously defined. Their data are entirely consistent with predicted behavior because the fraction of dieldrin and lindane removed in each successive extraction is approximately constant.

If kinetic limitations are possible for the sorption, they must be assumed probable for desorption as a consequence of Equation 2 and a large K_p. No studies of desorption kinetics have been reported, however. The only mention of desorption with respect to time is in the work of Paris et al.[40,42] in which the authors note that desorption equilibrium was reached within the time allowed for sorption equilibration.

C. Effect of Biomass and Solute Concentrations

The effect of changes in biomass and solute concentration is most easily seen if

Table 3
SORPTION AND DESORPTION BY A MIXED BACTERIAL POPULATION*

Compound	Concentration (μg/l)	Sorption (S) or desorption (D)	K_p
Benzo (a) pyrene	1.01	S	$(33.6 \pm 6.3) \times 10^4$
	1.01	D	$(36.9 \pm 7.0) \times 10^4$
	0.55	S	$(44.8 \pm 5.4) \times 10^4$
	0.55	D	$(49.5 \pm 5.0) \times 10^4$
Mirex	0.05	S	$(4.8 \pm 0.5) \times 10^5$
	0.05	D	$(3.3 \pm 0.5) \times 10^5$
Benz (a) anthracene	12.8	S	$(3.9 \pm 0.2) \times 10^4$
	12.8	D	$(2.9 \pm 0.3) \times 10^4$
	7.5	S	$(3.3 \pm 0.4) \times 10^4$
	7.5	D	$(2.5 \pm 0.2) \times 10^4$

* From Smith, J. H., Mabey, W. R., Bohonos, N., Holt, B. R., Lee, S. S., Chou, T.-W., Bomberger, D. C., and Mill, T., Rep. No. EPA-600/ 7-78-074, U.S. Environmental Protection Agency, Athens, Ga., May 1978.

Equation 1 is rewritten as

$$K_P = \frac{\frac{X}{m}}{\frac{X_o - X}{V}} \qquad (8)$$

where V is the mass of water containing the initial solute mass (X_o) and X is the mass of solute sorbed by biomass (m) at equilibrium. This equation alone is adequate to explain the effects observed by some workers[32,38,44,57] when biomass was varied. Certainly, partitioning according to Equation 8 is adequate to explain why the amount of solute per cell decreases with increasing biomass concentration without the necessity of a change in efficiency as suggested by Neudorf and Khan.[38]

An important point sometimes overlooked is the fact that concentrations used to calculate the accumulation factor (K_p) must be the equilibrium values X/m and (X_o − X)/V. Failure to use the equilibrium values can give results that are system specific and thus not comparable from one study to another. Such results have been left out of Table 1 and account for many of the voids in the K columns. It is perhaps worth noting that the error due to use of X_o instead of (X_o − X) can be small when X is much less than X_o.

D. Use of Axenic Cultures

No clear cut rules can be given concerning the choice of single vs. mixed population studies. Several workers have reported using axenic cultures of algae.[39,41,44,49,50] It is sufficient here to simply note that when it is desirable to make comparisons between different kinds of organisms, pure axenic cultures should be used. The opposite is true when system or gross properties are to be assessed. The extreme examples of this are represented by microcosm studies[36,47,53] and measurements on seston.[41,42,60]

E. Use of Labeled Compounds

Use of labeled compounds is not a panacea and can present special problems in measuring pollutant accumulation. The difficulties arise from the fact that measure-

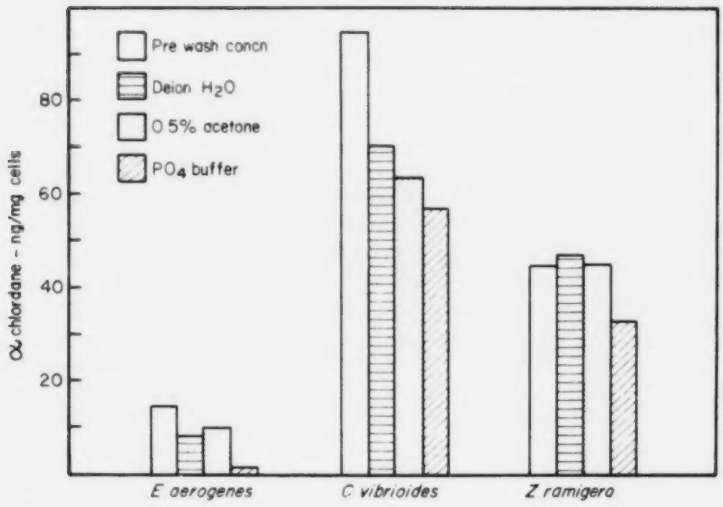

FIGURE 2. Persistence of α-chlordane on treated cells washed for 2 hr with deionized water, 0.5% aqueous acetone, and pH 7, 0.067 *M* phosphate (PO_4) buffer. (From Grimes, D. J. and Morrison, S. M., *Microb. Ecol.*, 2, 43, 1975. With permission.)

ment of radiation is not specific for the compound under study. This would be no problem if the compounds were completely pure and if some degradation did not occur under the condition of study. Labeled compounds, however, are not noted for high purity. It is easily seen from the partitioning equations that a small amount of impurity can cause a very large error in the concentration measurement if the partition coefficient of the impurity is markedly different from that of the study compound. In any event, the sacrifice of analytical specificity for sensitivity is to be undertaken only with caution and due safeguards.

F. Effect of Temperature

Only three studies[2,16,59] have examined the effect of temperature on the extent of partitioning. The first was with a euphausiid shrimp for which Cox[59] reported a Q_{10} of 1.2. The second study was with dead yeast[2] for which the ΔH was determined to be 5.2 kcal/mol. Finally, Smith et al.[16] found K_B for bacteria and methyl parathion to decrease from about 480 to 220 in going from 25°C to 2°C. In each case, the relatively small temperature coefficient is consistent with a physical process. It seems likely, therefore, that temperature will not be a major influence on passive bioaccumulation by organisms.

G. Environmental Relevance

In general, it appears that accumulation of hydrophobic compounds is similar for microbiota and higher organisms. Studies with microbiota, however, have so often involved washed cells and inadequate statistical treatment that it is difficult to determine whether reported differences are real or only experimental artifacts. For example, Reinert[43] reports about a tenfold lower accumulation factor for dieldrin in daphnid than in algae and Ellgehausen et al.[61] report similar results for DDT with daphnid and algae. In both cases, the accumulation factors are much lower than found with other organisms.[9]

Studies on phytoplankton,[9,61,65] zooplankton,[30,58,59] daphnid,[26,43] and protozoa[59] are qualitatively similar in behavior. Thus Cox[59] reports that initial uptake of DDT by a

euphausiid shrimp is unrelated to activity or respiration because heat killed animals had the same total uptake as live animals. He also notes that short term uptake was a linear function of concentration.

Several studies support the view that equilibration rates are lower for larger organisms[26,43,59,61] than for microbiota. This is consistent with expectations based on mass transport limitations.

It is interesting to compare the results of a large field study on PCBs in zooplankton[58] with laboratory studies. The field study shows a value of about 8×10^4 for K_B of the marine plankton whereas values reported for microorganisms are $(2.5 - 3.5) \times 10^3$, [29] $(5 - 17) \times 10^3$, [36] $(5 - 20) \times 10^3$, [49] and $(3 - 8) \times 10^3$ [50] (See Table 1). The results are similar in order of magnitude but accurate comparisons are not possible. PCBs are particularly difficult to compare because of analytical difficulties and the different ways in which concentrations are calculated from chromatographic data.

Two aspects of bioaccumulation that have received only slight attention are variation in K_B for different organisms and methods for predicting the K_B. We have noted earlier that some authors have tried to deal with species variation by relating K_B to lipid content[27,41,58] or organic carbon content.[58] Few data exist to support either approach for microorganisms but certainly the presumed importance of lipid in bioaccumulation is not new.

The correlation with organic carbon[58] is interesting because soil scientists often normalize partition coefficients to the organic carbon content of soils to reduce the variation in partition coefficients for different soils or sediments.[66] Also, several studies[4,28,57] have speculated about the role of soil microbiota in sorption of pollutants by soil. In fact, Ko and Lockwood[57] have suggested that sorption by microorganisms may contribute to the persistence and leaching resistance of pesticides such as DDT, dieldrin, and PCNB. It is also interesting that K_Bs for soil and bacteria are often quite similar.[16,60,67]

The problem of predicting bioaccumulation K_B can be approached in several ways. As noted earlier a reasonable correlation exists between partition coefficients and solubility and this has been used[36,62] to predict bioaccumulation. Partition coefficients should correlate better with each other than with solubility, however. Briggs[66] and Karickhoff et al.[64] have recently shown this for octanol-water (K_{ow}) and sediment-water partition coefficients, and Ellgehausen et al.[61] correlated K_{ow} with bioaccumulation by algae, daphnids, and catfish. Following their examples, data from Table 2 are plotted in Figure 1. A good correlation is obtained although the data come from a variety of sources and many of the K_{ow} values were calculated.

Table 4 shows five correlation equations for accumulation of organics from water by bacteria, fish, sediments, and a composite for algae, daphnia, and fish. The question that comes to mind on examining the equations is why the slope approximately equals 1 in four of the five cases when the parameters are so diverse. The slope is probably low in the last equation because of a low value used by the authors[61] for DDT.

It seems likely that the answer to this question lies in viewing the Ks as approximating equilibrium partition coefficients expected for a three phase system consisting of water, octanol, and biota. For any such system

$$K_B/K_{ow} = \frac{[P]_{Biota}}{[P]_{Octanol}} = K_{Bow} \qquad (9)$$

$$\log K_B - \log K_{ow} = \log K_{Bow} \qquad (10)$$

and so the slope $\frac{d \log K_B}{d \log K_{ow}}$ should equal one.

Table 4
EQUATION FOR ACCUMULATION OF ORGANICS FROM WATER

Equation	r^2	Ref.
$\log K_p = 0.907 \log K_{ow} - 0.361$	0.954	Fig. 1, this work
$\log BF = 0.837 \log K_{ow} - 0.770$[a]		62
$\log EM = 1.16 \log K_{ow} - 0.75$[b]		36
$\log K_{oc} = 1.0 \log K_{ow} - 0.21$[c]	1.00	64
$\log K = 0.49 \log K_{ow} + C$[d]	0.93 — 0.97	61

[a] BF is bioconcentration factor for fish. Obtained by substitution of equation in Reference 62.
[b] EM is ecological magnification in fish.
[c] K_{oc} is partition coefficient (between sediment and water) divided by organic carbon content of sediment.
[d] Slope is average value for algae, daphnia, and catfish. Actual values were 0.45, 0.50, 0.53, respectively.

The ultimate utility of such relationships and their reliability must await data of higher precision and studies using a greater diversity of organisms and compounds.

Environmental scientists are increasingly searching for methodology useful in predicting environmental impact, i.e., the use of "model ecosystems."[36] The "ecological magnification" for Metcalf's[36,53] model ecosystem amounts to a K_p except that the sorbing species, *Oedogonium candiacum*, is really an alga in association with other microbiota. In principle at least, this methodology should be reproducible given careful analysis and due consideration of possible kinetic limitations. Results from several studies of this type are included in Table 1,[36,47,53] but detailed discussion of such methods is beyond the scope of this review. It is important to remember, however, that the very act of measuring partitioning by a biological community implies the assumption that the results can be extrapolated to partitioning by other organisms or communities. This, of course, further implies that K_p is relatively independent of species and thus also of population diversity. The utility of this assumption is obvious to all in the environmental field, but it should be clear from this review that the basis for the conclusion is tentative at best rather than scientifically rigorous.

Attempts to assess the error in prediction of environmental partitioning from laboratory data can be found in the work of Paris et al.[40,42,60] They measured partition coefficients of pesticides[40,42] and PCBs[60] for various organisms both with pure cultures and mixed populations from natural sources. They found reasonably constant values (less than tenfold variation) for the K_p regardless of the source of the organisms. They also reported that the organisms from a given site accumulate only slightly more than seston from the same site.[60] Unfortunately, such data are not available for a much larger cross section of organisms, waters, and compounds.

VI. CONCLUSIONS

It seems likely that microbial accumulation of hydrophobic organics results from their large activity coefficients (fugacity) that also promote expulsion from the dissolved state by physical processes such as sediment sorption and volatilization. The partitioning mechanism is apparently passive in that it occurs to about the same extent or perhaps a little greater with dead cells than with live cells. Existing data are consistent with a simple equilibrium partition coefficient to quantitatively describe the extent

of accumulation. This is consistent with recent results showing that a pentachlorobiphenyl can readily be accumulated by marine algae suspended in solution with particulate materials having sorbed PCB,[55] a result entirely consistent with simple partitioning.

It is not clear whether sorbed materials reside in or on the cells or cell membranes. Nor have the kinetics of sorption or desorption been defined although it seems that these processes occur faster with smaller organisms.[42,61] Relationships between kinetics and properties of the compound have not been studied.

Available data suggest that it may be possible to predict accumulation through correlation with the octanol-water partition coefficient.[61] Also it is possible that differences resulting from organism diversity can be greatly reduced by normalization of partition coefficients to a lipid or organic carbon basis.

Use of microbial accumulation data for prediction of environmental behavior lacks a broad quantitative basis. It has much to recommend it, however, in terms of general descriptive precedent and practical, simple methodology. The soundness of this practice cannot be assessed until reliable K_{ow}s and K_Bs are measured to permit assessment of the variation in bioaccumulation due to species. To this end, it is fully as important to assess the precision of a measured parameter (K_B) as it is to assess its absolute magnitude. Thus absence of statistical treatment of data is a serious shortcoming of most studies reviewed here.

Currently available methodology is adequate to permit measurement of water-microbiota partition coefficients with good precision if reasonable precautions are observed. The most important limitation to credibility and precision in studies to date is the washing of cells and filtration of media.

Finally, it seems reasonable that the partition coefficient K_B should be viewed tentatively as a fundamental system property. Thus an important objective should be to show *not* how different K_B is for each organism, but rather to see how constant it is across many species and compounds.

APPENDIX
LIST OF COMPOUNDS IN TEXT

Common or trade name	Chemical designation
Aldrin	1,2,3,4,10,10-Hexachloro-1a, 4β, 4aβ, 5β, 8β, 8aβ-hexahydro-1,4:5,8-dimethanonaphthalene
Anthracene	Anthracene
Aroclor 1016	Mixture of polychlorinated biphenyl isomers
Aroclor 1242	Mixture of polychlorinated biphenyl isomers
Atrazine	2 Chloro-4-ethylamino-6-iosopropyl amine-s-triazine
Benz (*a*) anthracene	Benz (*a*) anthracene
Benzo (*a*) pyrene	Benzo(*a*)pyrene
benzo(*f*)quinoline	Benzo(*f*)quinoline
Benzo(*b*)thiophene	Benzo(*b*)thiophene
Chlorinated naphthalene	Chlorinated naphthalene
Chlordane	1,2,4,5,6,7,8,8-Octachloro-3a,4,7,7a-tetrahydro-4,7 methanoindan
DDE	1,1-Dichloro-2,2-*bis*(*p*-chlorophenyl) ethylene
DDT	1,1,1-Trichloro-2,2-*bis*(*p*-chlorophenyl) ethane
Dieldrin	3,4,5,6,9,9-Hexachloro-1aβ,2β,2aβ,3β,6β,6aβ,7β,-7aβ-octahydro-2,7:3,6 dimethanonaphth[2,3-b]oxirene
Dibenzothiophene	Dibenzothiophene

7H Dibenzo(*c*,*g*)-carbazole	7H Dibenzo(*c*,*g*)-carbozole
2,4-D	2,4 Dichlorophenoxyacetic acid
Decachlorobiphenyl	Decachlorobiphenyl
2,4-DBE	Butoxyethyl ester of 2,4-dichlorophenoxyacetic acid
Endrin	1,2,3,4,10,10-Hexachloro-6,7-epoxy -1,4,4a,5,6,7,8,8a-octa-hydro-endo, endo-1,4:5,8-dimethanonaphthalene
Heptadecane	Heptadecane
Hexadecane	Hexadecane
Hexadec-1-ene	Hexadec-1-ene
Hexachlorobenzene	Hexachlorobenzene
Heptachlor epoxide	1,4,5,6,7,8,8a-Heptachloro-2,3-epoxy-3a,4,7,7a-tetrahydro-4,7-methan-oindan
HCH (Lindane)	1,2,3,4,5,6,-Hexachlorocyclohexane
Methoxychlor	1,1,1-Trichloro-2,2-*bis*(*p* methoxyphenyl)-ethane
Methyl parathion	O,O-Dimethyl O-*p*-nitrophenyl phosphorothionate
Mirex	Dodecachlorooctahydro-1,3,4-metheno-2H-cyclobuto[*c*,*d*]pentalene
Octachlorobiphenyl	Octachlorobiphenyl
Parathion	O,O-Diethyl O-*p*-nitrophenyl phosphorothioate
PCB	Polychlorinated biphenyls
PCNB	Pentachloronitrobenzene
Pentadecane	Pentadecane
2,5,2′4′5′ Pentachlorobiphenyl	2,5,2′4′5′ Pentachlorobiphenyl
Photodieldrin	2a,3,3,4,5,5a-Hexachlorodecahydro-2,4,6-metheno-2H-cyclopenta [4,5]pentaleno[1,2-b] oxirene
2,5,2′-Trichlorobiphenyl	2,5,2′-Trichlorobiphenyl
2,5,2′,5′-Tetrachlorobiphenyl	2,5,2′,5′-Tetrachlorobiphenyl
Toxaphene	Chlorinated camphenes (mostly polychlorobornanes and polychlorobornenes and/or polychlorotricyclanes)

REFERENCES

1. **Ware, G. W. and Roan, C. C.,** Interaction of pesticides with aquatic microorganisms and plankton, in *Residue Reviews*, Vol. 33 (No. 15), Gunther, F. A., Ed., Springer-Verlag, New York, 1970.
2. **Herbes, S. E.,** Partitioning of polycyclic aromatic hydrocarbons between dissolved and particulate phases in natural waters, *Water Res.*, 11, 493, 1977.
3. **Hill, D. W. and McCarty, P. L.,** Anaerobic degradation of selected chlorinated hydrocarbon pesticides, *J. Water Pollut. Control Fed.*, 39, 1259, 1967.
4. **Shin, Y-O., Chodan, J. J., and Wolcott, A. R.,** Adsorption of DDT by soils, soil fractions, and biological materials, *J. Agric. Food Chem.*, 18, 1129, 1970.
5. **Voerman, S. and Tammes, P. M. L.,** Adsorption and desorption of lindane and dieldrin by yeast, *Bull. Environ. Contam. Toxicol.*, 4, 271, 1969.
6. **Ueyama, A., Egawa, H., and Tsuda, M,** Absorption of γ-BHC by *Fusarium roseum* link in culture, *Ann. Phytopath. Soc. Jpn.*, 35, 347, 1969.
7. **Walsh, G. E., Ainsworth, K. A., and Faas, L.,** Effects and uptake of chlorinated naphthalenes in marine unicellular algae, *Bull. Environ. Contam. Toxicol.*, 18, 297, 1977.
8. **Wheeler, W. B.,** Experimental absorption of dieldrin by *Chlorella*, *J. Agric. Food Chem.*, 18, 416, 1970.
9. **Cox, J. L.,** Low ambient level uptake of ^{14}C-DDT by three species of marine phytoplankton, *Bull. Environ. Contam. Toxicol.*, 5, 218, 1970.
10. **Sugiura, K., Sato, S., and Goto, M.,** Adsorption-diffusion mechanism of BHC-residues. A consideration based on bacteria experiments as models, *Chemosphere*, 4, 189, 1975.
11. **Leshniowsky, W. O., Dugan, P. R., Pfister, R. M., Frea, J. I., and Randles, C. I.,** Aldrin: removal from lake water by flocculent bacteria, *Science*, 169, 993, 1970.

12. **Sodergren, A.**, Uptake and accumulation of C^{14}-DDT by *Chlorella* sp. (Chlorophyceae), *Oikos.*, 19, 126, 1968.
13. **Kennedy, R. W., Finnerty, W. R., Sudarsanan, K., and Young, R. A.**, Microbial assimilation of hydrocarbons. I. The fine-structure hydrocarbon oxidizing *Acinetobacter* sp., *Arch. Microbiol.*, 102, 75, 1975.
14. **Kennedy, R. S. and Finnerty, W. R.**, Microbial assimilation of hydrocarbons. II. Intracytoplasmic membrane induction in *Acinetobacter* sp., *Arch. Microbiol.*, 102, 85, 1975.
15. **Poglazova, M. N. and Meisel, M. N.**, Localization of benz(a)pyrene in bacterial cells, *Mikkrobiologija*, 40, 913, 1971.
16. **Smith, J. H., Mabey, W. R., Bohonos, N., Holt, B. R., Lee, S. S., Chou, T. -W., Bomberger, D. C., and Mill, T.**, Environmental Pathways of Selected Chemicals in Freshwater Systems. Part II. Laboratory Studies, Rep. No. EPA-600/7-78-074, U.S. Environmental Protection Agency, Athens, Ga., May 1978.
17. **Ludvic, J., Munk, V., and Dostalek, M.**, Ultrastructural changes in the yeast *Candida lipolytica* caused by penetration of hydrocarbons into the cell, *Experientia*, 24, 1066, 1968.
18. **Diamond, J. M. and Wright, E. M.**, Molecular forces governing non-electrolyte permeation through cell membranes, *Proc. Roy. Soc. London Ser. B.*, B172, 273, 1969.
19. **Diamond, J. M. and Wright, E. M.**, Biological membranes: the physical basis of ion and nonelectrolyte selectivity, *Ann. Rev. Physiol.*, 31, 581, 1969.
20. **Davson, H. and Danielli, J. F.**, *The Permeability of Natural Membranes*, Cambridge University Press, London, 1952.
21. **Kotyk, A. and Janacek, K.**, *Cell Membrane Transport*, 2nd ed., Plenum Press, New York, 1975.
22. **Stein, W. D.**, *The Movement of Molecules Across Cell Membranes*, Academic Press, New York, 1967.
23. **Jackson, M. J. and Cohn, V. H.**, Determinants of xenobiotic transport at biological barriers, in *Handbook of Physiology*, Waverly Press, Baltimore, 1977.
24. **Tanford, C.**, The hydrophobic effect and the organization of living matter, *Science*, 200, 1012, 1978.
25. **Lakowicz, J. P., Hogen, D., and Omann, G.**, Diffusion and partitioning of a pesticide, lindane, into phosphatridylcholine bilayers, *Biochim. Biophys. Acta*, 471, 401, 1977.
26. **Canton, J. H. Greve, P. A., Slooff, W., and van Esch, G. J.**, Toxicity, accumulation, and elimination studies of α-hexachlorocyclohexane (αHCH) with freshwater organisms of different trophic levels, *Water Res.*, 9, 1163, 1975.
27. **Canton, J. H., van Esch, G. J., Greve, P. A., and van Hellemond, A. B. A. M.**, Accumulation and elimination of α-hexachlorocyclohexane (α-HCH) by the marine algae *Chlamydomonas* and *Dunaliella*, *Water Res.*, 11, 111, 1977.
28. **Chacko, C. I. and Lockwood, J. L.**, Accumulation of DDT and dieldrin by microorganisms, *Can. J. of Microbiol.*, 13, 1123, 1967.
29. **Dive, D., Erb, R., Leclerc, H., Priem, M. N., and Colein, M. Ph.**, Toxicite et bioaccumulation d'isomeres de polychlorobiphernyles par le protozoaire cilie *Colpidium campylum* (Stokes), *Eur. J. Toxicol.*, 9, 105, 1976.
30. **Hamelink, J. L. and Waybrant, R. C.**, DDE and lindane in a large-scale model lentic ecosystem, *Trans. Am. Fish. Soc.*, 105, 124, 1976.
31. **Hollister, T. A., Walsh, G. E., and Forester, J.**, Mirex and marine unicellular algae: accumulation, population growth, and oxygen evolution, *Bull. Environ. Contam. Toxicol.*, 10, 753, 1975.
32. **Johnson, B. T. and Kennedy, J. O.**, Biomagnification of p,p'-DDT and methoxychlor by bacteria, *Appl. Microbiol.*, 26, 66, 1973.
33. **Keil, J. E. and Priester, L. E.**, DDT uptake and metabolism by a marine diatom, *Bull. Environ. Contam. Toxicol.*, 4, 169, 1969.
34. **King, P. H., Yeh, H. H., Warren, P. S., and Randall, C. W.**, Distribution of pesticides in surface waters, *J. Am. Water Works Assoc.*, 61, 483, 1969.
35. **Kurtz, D. A.**, Adsorption of PCBs and DDTs on membrane filters — a new analysis method, *Bull. Environ. Contam. Toxicol.*, 17, 391, 1977.
36. **Metcalf, R. L., Sanborn, J. R., Lu, P-Y., and Nye, D.**, Laboratory model ecosystem studies of the degradation and fate of radiolabeled tri-, tetra-, and pentachlorobiphenyl campared with DDE, *Arch. Environ. Contam. Toxicol.*, 3, 151, 1975.
37. **Grimes, D. J. and Morrison, S. M.**, Bacterial bioconcentration of chlorinated hydrocarbon insecticides from aqueous systems, *Microb. Ecol.*, 2, 43, 1975.
38. **Neudorf, S. and Khan, M. A. W.**, Pick-up and metabolism of DDT, dieldrin, and photodieldrin by a freshwater alga (*Ankistrodesmus Amalloides*) and a microcrustacean (*Daphnia Pulex*), *Bull. Environ. Contam. Toxicol.*, 13, 443, 1975.
39. **O'Kelley, J. C. and Deason, T. R.**, Degradation of Pesticides by Algae, Rep. No. EPA-600/3-76-022, U.S. Environmental Protection Agency, Athens, Ga., March 1976.

40. **Paris, D. F., Lewis, D. L., and Barnett, J. T.,** Bioconcentration of toxaphene by microorganisms, *Bull. Environ. Contam. Toxicol.*, 17, 564, 1977.
41. **Paris, D. F., Lewis, D. L., Barnett, J. T., and Baughman, G. L.,** Microbial Degradation and Accumulation of Pesticides in Aquatic Systems, Rep. No. EPA-660/3-75-007, U.S. Environmental Protection Agency, Athens, Ga., March 1975.
42. **Paris, D. F. and Lewis, D. L.,** Accumulation of methoxychlor by microorganisms isolated from aqueous systems, *Bull. Environ. Contam. Toxicol.*, 15, 24, 1976.
43. **Reinert, R. E.,** Accumulation of dieldrin in an alga (*Scenedesmus obliquus*), *Daphnia magna*, and the guppy (*Poecilia reticulata*), *J. Fish. Res. Board Can.*, 29, 1413, 1972.
44. **Rice, C. P. and Sikka, H. C.,** Uptake and metabolism of DDT by six species of marine algae, *J. Agric. Food Chem.*, 21, 148, 1973.
45. **Rice, C. P. and Sikka, H. C.,** Fate of dieldrin in selected species of marine algae, *Bull. Environ. Contam. Toxicol.*, 9, 116, 1973.
46. **Rose, F. L. and McIntire, C. D.,** Accumulation of dieldrin by benthic algae in laboratory streams, *Hydrobiologia*, 35, 481, 1970.
47. **Sanborn, J. R., Metcalf, R. L., Bruce, W. N., and Lu, P-Y.,** The fate of chlordane and toxaphene in a terrestrial-aquatic model ecosystem, *Environ. Entomol.*, 5, 533, 1976.
48. **Schauberger, C. W. and Wildman, R. B.,** Accumulation of aldrin and dieldrin by blue-green algae and related effects on photosynthetic pigments, *Bull. Environ. Contam. Toxicol.*, 17, 534, 1977.
49. **Urey, J. C., Kricher, J. C., and Boylan, J. M.,** Bioconcentration of four pure PCB isomers by *Chlorella pyrenoidosa*, *Bull. Environ. Contam. Toxicol.*, 16, 81, 1976.
50. **Valentine, J. P. and Bingham, S. W.,** Influence of algae on amitrole and atrazine residues in water, *Can. J. Bot.*, 54, 2100, 1976.
51. **Vance, B. D. and Drummond, W.,** Biological concentration of pesticides by algae, *J. Am. Water Works, Assoc.* 61, 360, 1969.
52. **Lorbacker, H., Puls, H. -D., and Schlipkoter, H. -W.,** Studies of the storage and metabolism of 3,4-benzpyrene (benxo-(a)-pyrene) in microorganisms, *Zentralbl. Bakteriol. Para Sitenk. Infektionski. Hyg. Abt. Orig. Reihe B*, 155, 168, 1971.
53. **Metcalf, R. L., Kapoor, I. P., Lu, P-Y., Schuth, C. K., and Sherman, P.,** Model ecosystem studies of the environmental fate of six organochlorine pesticides, *Environ. Health Perspectives*, 4, 35, 1973.
54. **Horitsu, H., Sugiura, M., and Omoeda, M. T.,** On absorptive behavior of benzo(a)pyrene by yeast and bacteria, *Hakko Kogaku Zasshi*, 55, 51, 1977.
55. **Harding, L. W. and Phillips, J. H.,** Polychlorinated biphenyls: transfer from microparticulates to marine phytoplankton and the effects of photosynthesis, *Science*, 202, 1189, 1978.
56. **Karickhoff, S.,** *Contaminants and Sediments*, Vol. 2, Yen, T. F., Ed., Ann Arbor Press, Ann Arbor, 1980, in press.
57. **Ko, W. H. and Lockwood, J. L.,** Accumulation and concentration of chlorinated hydrocarbon pesticides by microorganisms, *Can. J. Microbiol.*, 14, 1075, 1968.
58. **Clayton, J. R., Pavlou, S. P., and Breitner, N. F.,** Polychlorinated biphenyls in coastal marine zooplankton: bioaccumulation by equilibrium partitioning, *Environ. Sci. Technol.*, 11, 676, 1977.
59. **Cox, J. L.,** Uptake, assimilation, and loss of DDT residues by *Euphausia Pacifica*, a euphausiid shrimp, *Fish. Bull.*, 69, 627, 1971.
60. **Paris, D. F., Steen, W. C., and Baughman, G. L.,** Role of physico-chemical properties of aroclors 1016 and 1242 in determining their fate and transport in aquatic environments, *Chemosphere*, 7, 319, 1978.
61. **Ellgehausen, H., Guth, J. A., and Esser, H. O.,** Factors determining the bioaccumulation potential of pesticides in the individual compartment of aquatic food chains, in *Proc. 4th Int. Congr. Pesticide Chemistry (IUPAC)*, Zurich, 1978, in press.
62. **Choiu, C. T., Freed, V. H., Schmedding, D. W., and Kohnert, R. L.,** Partition coefficient and bioaccumulation of selected organic chemicals, *Environ. Sci. Technol.*, 11, 475, 1977.
63. **Leo, A. J.,** Calculation of partition coefficients useful in the evaluation of the relative hazards of various chemicals in the environment, in Structure-Activity Correlations in Studies of Toxicity and Bioconcentration with Aquatic Organisms, Veith, G. D. and Konasewich, D. G., Eds., International Joint Commission, 1975.
64. **Karickhoff, S. W., Brown, D. S., and Scott, T. A.,** Sorption of hydrophobic pollutants on natural sediments, *Water Res.*, 13, 241, 1979.
65. **Menzel, D. W., Anderson, J., and Randtke, A.,** Marine phytoplankton vary in their response to chlorinated hydrocarbons, *Science*, 167, 1724, 1970.
66. **Briggs, G. G.,** A simple relationship between soil of organic chemicals and their octanol/water partition coefficients, in Proc. 7th British Insecticide and Fungicide Conf., A.W. Billet, Worcester, England, 1973, 475.
67. **Karickhoff, S. W. and Steen, W. C.,** personal communication, 1979.

68. **W. C. Steen,** personal communication.
69. **G. D. Veith,** personal communication.
70. **Lieb, W. R. and Stein, W. D.,** Biological membranes behave as non-porous polymeric sheets with respect to the diffusion of non-electrolytes, *Nature (London),* 224, 240, 1969.